

AMENDMENT TO THE CLAIMS

Claim 1. (Currently amended) A process for obtaining osteogenic proteins from mammalian bone tissue comprising:

contacting bone tissue with an acidic demineralization medium to provide demineralized bone tissue and a ~~mineralized supernatant solution~~ mineral-containing supernatant;

separating the ~~mineralized supernatant solution~~ mineral-containing supernatant from the demineralized bone tissue;

removing at least part of the mineral component of the ~~mineralized supernatant solution~~ mineral-containing supernatant {by contacting the ~~mineralized supernatant solution~~ mineral-containing supernatant with a mineral precipitation agent} to provide a protein supernatant solution;

extracting osteogenic proteins from the protein supernatant solution by contacting the protein supernatant solution with a protein extraction agent to provide an extracted protein medium; and

recovering osteogenic proteins from the extracted protein medium.

Claim 2. (Original) The method of claim 1 wherein said recovering step comprises

filtering said extracted protein medium in a first ultrafiltration step using a first ultrafiltration membrane having a nominal molecular weight cutoff corresponding to a high molecular weight limit to provide a permeate comprising a first osteogenic solution;

filtering the first osteogenic solution in a second ultrafiltration step using a second ultrafiltration membrane having a nominal molecular weight cutoff corresponding to a low molecular weight limit to provide a retentate comprising a second osteogenic solution; and

purifying the osteogenic proteins in said second osteogenic solution.

Claim 3. (Original) The method of claim 2 wherein said protein extraction agent comprises guanidine hydrochloride.

Claim 4. (Original) The method of claim 3 wherein said purifying step comprises removing said guanidine hydrochloride by at least one diafiltration step in which the osteogenic proteins are diafiltered into a diafiltration medium that does not comprise guanidine hydrochloride.

Claim 5. (Original) The method of claim 4 wherein said purifying step further comprises at least one purification operation selected from the group consisting of lyophilization and precipitation.

Claim 6. (Original) The method of claim 3 wherein said purifying step comprises a first diafiltration step in which at least a portion of the guanidine hydrochloride is removed by diafiltering the osteogenic protein into a first diafiltration medium comprising urea, and a second diafiltration step in which at least a portion of the urea is removed by diafiltering the osteogenic protein into a second diafiltration medium comprising dilute hydrochloric acid.

Claim 7. (Original) The method of claim 6 wherein said purifying step further comprises lyophilizing the proteins from the second diafiltration medium to provide a solid osteogenic protein mixture.

Claim 8. (Original) The method of claim 7 wherein said purifying step further comprises dissolving said solid osteogenic protein mixture in a first purification medium comprising dilute hydrochloric acid; precipitating the proteins by contacting the first purification medium with a protein precipitating agent; separating the precipitated proteins from the first purification medium and the protein precipitating agent; and

dissolving the separated and precipitated proteins in a second purification medium comprising dilute hydrochloric acid; and
lyophilizing the proteins from the second purification medium to provide solid osteogenic proteins.

Claim 9. (Currently amended) A method for isolating osteogenic proteins from mammalian bone tissue comprising:

demineralizing bone tissue in an acid medium to provide demineralized bone tissue and a mineral-containing acid supernatant;

removing at least a portion of the minerals from the mineral-containing acid supernatant to provide a protein supernatant solution;

extracting osteogenic proteins from the protein supernatant solution with a protein extraction agent to provide an extracted protein medium; and

recovering osteogenic proteins from the extracted protein medium.

Claim 10. (Original) The method of claim 9 wherein the acid medium comprises hydrochloric acid.

Claim 11. (Currently amended) The method of claim 9 wherein said removing step comprises contacting the mineralized mineral-containing acid supernatant solution with a mineral precipitation agent.

Claim 12. (Original) The method of claim 11 wherein the mineral precipitation agent comprises calcium oxalate.

Claim 13. (Original) The method of claim 9 wherein said extracting step comprises contacting said protein supernatant solution with guanidine hydrochloride.

Claim 14. (Original) The method of claim 9 wherein said recovering step comprises

filtering said extracted protein medium in a first ultrafiltration step to remove proteins having a molecular weight exceeding a desired high molecular weight limit to provide a first filtered solution;

filtering the first filtered solution in a second ultrafiltration step to remove proteins having a molecular weight below a desired low molecular weight limit to provide a second filtered solution; and

purifying the osteogenic proteins in said second filtered solution.

Claim 15. (Original) The method of claim 14 wherein said purifying step comprises removing said protein extraction agent by at least one diafiltration step in which the osteogenic proteins are transferred to a medium that does not comprise the protein extraction agent.

Claim 16. (Original) The method of claim 15 wherein said protein extraction agent comprises guanidine hydrochloride.

Claim 17. (Original) The method of claim 15 wherein said protein extraction agent comprises urea.

Claim 18. (Original) The method of claim 15 wherein said purifying step comprises a first diafiltration step in which the osteogenic proteins are transferred to a medium that does not comprise the protein extraction agent, and a second diafiltration step in which the osteogenic proteins are transferred to a dilute acid medium that does not comprise the protein extraction agent.

Claim 19. (Original) The method of claim 15 wherein said purifying step further comprises at least one purification operation selected from the group consisting of lyophilization and precipitation.

Claim 20. (Original) The method of claim 14 wherein said protein extraction agent comprises guanidine hydrochloride and said purifying step comprises

a first diafiltration step in which the guanidine hydrochloride is removed by diafiltering the osteogenic protein into a first diafiltration medium comprising urea, and
a second diafiltration step in which the urea is removed by diafiltering the osteogenic protein into a second diafiltration medium comprising dilute hydrochloric acid.

Claim 21. (Original) The method of claim 20 wherein said purifying step further comprises

lyophilizing the proteins from the second diafiltration medium to provide solid osteogenic proteins.

Claim 22. (Original) The method of claim 21 wherein said purifying step further comprises

dissolving said solid osteogenic proteins in a first purification medium comprising dilute hydrochloric acid;

precipitating the proteins by contacting the first purification medium with a protein precipitating agent;

separating the precipitated proteins from the first purification medium and the protein precipitating agent; and

dissolving the separated and precipitated proteins in a second purification medium comprising dilute hydrochloric acid; and

lyophilizing the proteins from the second purification medium to provide purified osteogenic proteins.

Claim 23. (Original) The method of claim 22 wherein said protein precipitating agent comprises acetone.

Claim 24. (Currently amended) A method for isolating osteogenic proteins from mammalian bone tissue comprising:

demineralizing bone tissue in an acid medium to provide demineralized bone tissue and a mineral-containing acid supernatant;

extracting osteogenic proteins from the ~~protein-supernatant solution~~ mineral-containing acid supernatant with a protein extraction agent to provide an extracted protein medium; and

recovering osteogenic proteins from the extracted protein medium.